Agr. Nat. Resour. 55 (2021) 1023–1038

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Biochemical and physiological traits of strawberry as influenced by organic acids and deficit irrigation under colored netting

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Article Info

Article history:
Received 10 August 2021
Revised 9 November 2021
Accepted 12 November 2021
Available online 28 December 2021

Keywords:
Antioxidant capacity,
Colored netting,
Drought stress,
Organic acid,
Taste index

Abstract

To improve strawberry fruit quantity and quality, the effects of colored netting and foliar application of amino acids and humic acid were studied on the physiological traits of strawberries exposed to different irrigation frequencies in a factorial experiment in a greenhouse of the Lahijan Agricultural Research Station, Iran. The studied factors were colored nets at four levels (no netting, green netting, red netting, yellow netting), organic acids at four levels (water as the control, humic acid, glutamine, arginine), and irrigation at three levels (irrigation intervals of 2 d, 4 d and 6 d). The highest phenolics content, antioxidant capacity, total soluble solids, taste index and membrane stability index (MSI) were obtained using colored netting. Among the organic acid treatments, the plants treated with humic acid had the highest proline content (mean ± SD; 0.82 ± 0.07 mM/L) and MSI (79.89 ± 2.1%) and those treated with glutamine had the highest yield (14.48 ± 1.44 g/plant) and stability index (79.91 ± 2.11%). The irrigation intervals of 2d and 4 d had an optimal effect on the studied traits compared to 6 d. The results on the three-way interaction effects of the trial factors revealed that 'green netting × glutamine × 4 days' produced the highest fruit yield (40.80 ± 2.41 g/plant), 'no netting × humic acid × 6 days' was related to the highest antioxidant capacity (84.39 ± 4.23% of 2,2-diphenylpicrylhydrazyl), 'no netting × control × 4 days' was related to the highest peroxidase content (0.085 ± 0.003 units) and 'red netting × control × 4 days' and 'red netting × humic acid × 2 days' were related to the highest fruit protein content.

Introduction

The strawberry is a perennial herbaceous plant species from the genus *Fragaria* that lives for an average of 3–5 yr (JaliliMarandi, 2010). It is an economically important plant with great nutritional value, a popular flavor and vitamins-rich contents (Fatemi et al., 2009). Strawberry plants are very sensitive to moisture deficiency due to their shallow root system; therefore, drought stress can have substantial effects on the strawberry fruit yield and quality in regions with tropical and dry summers and limited water resources (Li et al., 2010).

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https://doi.org/10.34044/j.anres.2021.55.6.13

The use of shade netting has become a common agro-technological approach in the cultivation of ornamental plants (Shahak, 2008; Nissim-Levi et al., 2008), vegetables (Fallik et al., 2009) and fruit trees (Shahak et al., 2004; Shahak et al., 2008a). Colored shade nets that can filter sunlight are also associated with physical protection of the crop against sunlight, heat, drought, wind, hail and winged insects (Shahak et al., 2016). The materials used in the structure of these nets contribute to absorb ultraviolet (UV), blue, green, yellow, red and far-red spectra. In addition, the netting can change direct light into scattered light (Shahak et al., 2004). The application of colored shade nets improves fruit quality and increases commercial fruits (Ilić et al., 2015). The severe stimulating effect of yellow nets on fruits may be partially related to the increased level of bioactive gibberellins which has already been proven to increase with the irradiance of supplemental green light (Wang and Folta, 2013).

Drought is the most important and destructive abiotic stresses affecting plant growth and productivity in many areas (Klamkowski and Treder, 2008). The main processes in plants that are affected by abiotic stresses are photosynthesis, osmotic potential and stomatal conductance or a combination of these properties (Bushra et al., 2014). Drought and salinity stress in strawberries can delay the development of their reproductive organs, thereby reducing the number of flowers and fruits produced (Aghdam et al., 2013). Currently, the use of organic acids to improve the quantity and quality of agricultural and horticultural products is common practice (Samavat and Malakouti, 2005). Amino acids can be stored as a source of carbon and energy in plants and since arginine is a precursor to the synthesis of polyamines, its pre-harvest application increases phenolic compounds and improves quality parameters, including fruit firmness (Liu et al., 2006). Arginine application in beans is involved in stimulating the hormones responsible for flower and fruit production (Zeid, 2009), increasing the uptake of macronutrients and micronutrients (Sarropoulou et al., 2014) and the synthesis of proteins and enzymes (Bibi et al., 2010). It has been claimed that strawberries have a significant response to the use of humic acid due to their shallow root system and high fruit production rate in relation to plant size (Piccolo, 1996). It was reported that the application of humic acid to strawberries of the cultivar ‘Selva’ increased their leaf area, fruit weight, achene number, shoot and root fresh and dry weights and their yield (Farahi et al., 2013). In a study on strawberries, the application of humic acid-containing fertilizer significantly increased the yield compared to the use of chemical fertilizers (Shehata et al., 2011). In strawberries, the uptake of major plant nutrients is mediated by humic acid, with a stimulative effect of humic substances on plant growth being the enhanced uptake of macro nutrients (Eshghi and Garazhian, 2015). A positive effect was reported of humic acid on the percentage of fruit dry matter in strawberries (Ameri and Tehranifar, 2012). Based on these studies, the present study investigated improving strawberry fruit quantity and quality by changing the growth environment using colored nets and the foliar application of amino and humic acids on the morphological and physiological traits of strawberries under different irrigation frequencies.

Materials and Methods

Experimental design and trial traits

The research was conducted in a greenhouse of the Flower and Ornamental Plants Research Station of Lahijan, Iran as a factorial experiment based on a randomized complete block design with three factors, each at four levels. The first factor was assigned to shade netting colors (no netting as the control, green, red, yellow), the second factor was assigned to organic acids (water as the control, 100 mg/l humic acid, 100 mg/l glutamine, 100 mg/l arginine) and the third factor was assigned to irrigation interval (2 d, 4 d, 6 d). The experiment consisted of 48 treatments, 3 replications and 144 pots. Lahijan is a mountainous city located in the eastern part of Guilan province, Iran. Lahijan has a temperate and humid climate and is in the temperate Caspian region. The temperature and humidity ranges of the glasshouse were 24–32°C and 75–85%, respectively.

Cultural practices

Strawberry rooted runners were planted on 11 February 2020 in 3 L plastic pots containing leaf litter, garden soil and manure (1:1:1). The foliar application was performed in two stages, the first on 16 March 2020 and the second on 14 April 2020. The treatments were applied after the plants had produced 5–6 fully developed leaves. Foliar spraying treatments were performed at the stage of complete plant establishment (first stage) and the beginning of flowering stage (second stage). The experimental strawberry cultivar was Gaviota. Plant fertilizing was the same for all treatments during the experiment and was done twice with complete fertilizer at 1 mth and 2 mth after plant establishment. There were no pest, disease or weed problems during the experiment. If necessary, hand weeding was done. The shading nets were placed 2 m above the ground, from the beginning of experiment. At each irrigation interval, 150 mL/pot water was applied.

The recorded traits were: total fruit yield, phenolics, antioxidant capacity, peroxidase enzyme, membrane stability index (MSI), total soluble solids (TSS), taste index (TSS/titratable acidity, TA), fruit protein and proline. From the first fruit harvest to the end of the experiment, the fruits of each experimental pot were collected every week and weighed. Finally, the total fruit weight was calculated at the end of the experiment to obtain the fruit yield of each treatment.

Total polyphenols

The amount of total polyphenols of berries was determined using Folin-Ciocalteu reagent. A sample (1 g) of fruit was rasped in 10 mL methanol for 2 min and the solution was filtered. Then, 0.5 mL of diluted extract (1:10 g/L) was added with 5 mL diluted Folin-Ciocalteu and then with 4 mL sodium carbonate solution (7.5% volume per volume). The samples were kept at the laboratory temperature for 15 min and their absorbance was read at 765 nm using a spectrophotometer (McDonald et al., 2001). The total phenol contents were calculated using the equation for the gallic acid-absorbance graph obtained from absorbance measurements of gallic acid prepared at different concentrations at 765 nm. The standard curve was prepared on the basis of gallic acid concentrations of 0 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL and 0.5 mg/mL and linear equation was determined as $y = bx + a$, where absorption measurements were used for y to yield the concentration x (McDonald et al., 2001).

Antioxidant capacity and peroxidase

To measure antioxidant capacity, 1 g of the fruit was wrapped in a piece of foil and kept in liquefied nitrogen for 2–3 min. Then, it was ground with 10 mL 85% methanol. The samples were placed at room temperature for 1 hr before being filtered and then centrifuged at 3,000 revolutions per minute (rpm) for 5 min. Then, 150 mL of the supernatant was taken and 850 µL 2,2-diphenylpicrylhydrazyl (DPPH) was added. The resulting solution was shaken and kept in darkness for 20 min. After blank placement and an instrument reset, first only DPPH was poured into a coquette and read. The antioxidant capacity of the fruit extracts was calculated using a spectrophotometer at 517 nm as the percentage of DPPH inhibition according to Bursal and Gulcin (2011), as shown in Equation (1):

$$\%DPPH_{sc} = (A_{cont} - A_{samp}) / A_{cont} \times 100 \quad (1)$$

where $\%DPPH_{sc}$ is the percentage inhibition, A_{cont} is the DPPH absorbance and A_{samp} is the absorbance of the sample + DPPH.

To measure peroxidase (POD) activity, the extract was prepared and then the change in optical density 430 nm was recorded every 30 s for 2 min using a spectrophotometer (Chance and Maehly, 1995).

Membrane stability index

The membrane stability index (MSI) was measured using the leaf electrolyte leakage method and an electrical conductivity meter. The MSI was calculated according to Sairam et al. (2002) using Equation (2):

$$\text{Membrane stabiluty index} = [\,1 - (EC_1 - EC2)] \times 100 \quad (2)$$

where EC_1 is the initial electrical conductivity of fresh leaves and EC_2 is the electrical conductivity of the sample at normal laboratory temperature after having been stored in a freezer (-20°C) for 24 hr.

Total soluble solids and taste index

Total soluble solids (TSS) were measured using a Japanese E20-ATC-Atago refractometer in the range 0–20%. Fruits were transversely cut and 1–2 drops of the extract were dropped on the device and the TSS value was recorded. The taste index was defined as TSS divided by total acidity (TA).

Protein content

Protein was measured using the Bradford method. In brief, 100 µL of the extract was added to 5 mL of Bradford solution. After 30 min at laboratory conditions, the absorbance was read at 595 nm using a Shimadzu 160-UV spectrophotometer and the protein content was estimated in milligrams per gran using a standard curve (Askary et al., 2012).

Proline

Proline was measured using the method of Bates et al. (1973). A sample (0.1 g) of fresh tissue was ground in 10 mL of 3% sulfosalicylic acid solution to yield a uniform mixture. The resulting extract was centrifuged at 10,000 rpm using a NAPCO2028R centrifuge for 5 min. Then, 2 mL of the supernatant was mixed with 2 mg of ninhydrin reagent and 2 mL of pure acetic acid and placed at 100°C in a warm water bath for 1 hr. The upper colored layer, which contained toluene and proline, was used to measure the proline content. The absorbance of a certain amount of this layer was read at 520 nm and the proline content of the sample was determined based on a standard curve. The linear equation was $y = 0.129x + 0.009$, where y is the reading and x is the concentration of proline in millimolars per liter (mM/L).

Statistical analysis

After ensuring the data were normally distributed and consistent with the assumptions for analysis of variance, a three-way data analysis of variance was performed using the MSTATC software (Elekhtyar, 2018). The three experimental factors were shade netting at four levels, organic acids at four levels, and irrigation interval at three levels. The means were compared using the least significant difference and Duncan's new multiple range test at $p < 0.05$. Data were presented as the mean ± SD.

Results

The chemical properties of the studied substrate were determined. The organic matter, organic carbon, electrical conductivity (EC) and pH of the trial substrate were 5.89%, 3.4%, 0.52 dS/m and 7.6, respectively. The amounts of N, P and K in the substrate were 0.29%, 0.09 mg/L and 4.8 mg/L, respectively.

Total fruit yield

The total fruit yield was significantly affected by all factors and the two-way and three-way interactions of the experimental factors (Table 1). According to the comparison of means of main effects (Table 2), the highest and lowest fruit yields were obtained from yellow netting (14.72 ± 2.1 g/plant) and the control with no netting (9.74 ± 1.3 g/plant), respectively. Among the organic acids, the treatment of glutamine produced the highest yield (14.48 ± 1.44 g/plant), as shown in Table 3. Among the irrigation levels, the highest and lowest fruit yields were produced by the plants irrigated every 4 d (14.77 ± 2.24 g/plant) and those irrigated every 6 d (8.27 ± 2.71 g/plant), respectively (Table 4). The comparison of mean yields as affected by the interaction of 'netting × organic acid' showed that under no netting, the highest yield was for the application of all organic acids (humic, glutamine, arginine) and the lowest for the treatment with no application of organic acid (9.45 ± 1.17 g/plant), as shown in Fig. 1, whereas for the green, red and yellow netting colors, the highest yield was with glutamine (21.38 ± 2.24 g/plant), glutamine (12.97 ± 1.78 g/plant) and no application (13.26 ± 2.61 g/plant), respectively (Fig. 1). However, for the overall specific combinations of color of shade netting and application of organic acid, the crop under green netting with an application of glutamine had the highest yield (21.38 ± 2.24 g/plant). The interaction of 'netting × irrigation' showed that under no netting and red netting conditions, the highest yields were for the crops with irrigation intervals of 2 d (12.40 ± 1.86 and 11.32 ± 2.05 g/plant, respectively) and 4 d (12.09 ± 1.17 and 9.82 ± 2.13 g/plant, respectively), as shown in Fig. 2, whereas under the green netting, the crops with an irrigation interval of 4 d had the highest yield (18.68 ± 2.11 g/plant). However, there was no significant difference for strawberry yield among the irrigation intervals under yellow netting. Overall, for the combination treatment of color of netting and irrigation treatment, the crops under green netting and an irrigation interval of 4 d had the highest yield (18.68 ± 2.11 g/plant), as shown in Fig. 2. Based on the comparison of means for the interaction of "organic acid × irrigation" under no application, application of humic acid and application of arginine, the highest yields were for the irrigation intervals of 2 d and 4 d (12.86 ± 0.87 and 13.72 ± 1.52 g/plant, respectively), 4 d (10.62 ± 2.69 g/plant) and 4 d (14.90 ± 1.77 g/plant), respectively (Fig. 3), whereas under the application of humic acid, the highest yield was with an irrigation interval of 2 d (15.63 ± 2.49 g/plant). However, for the overall combination treatment of the application of organic acid and irrigation interval, the crops with an application of glutamine and an irrigation interval of 4 d had the highest yield (19.85 ± 1.64 g/plant), as shown in Fig. 3. For the 3- way interaction, the mean yield interactions between the two factors of organic acid applications and irrigation intervals under each color of netting are presented in Fig 4. Under red and yellow netting and the application of organic acid, the crop yield did not respond to irrigation interval (Figs. 4C and 4D). In no and green netting types, the crops responded differently in patterns and rate (Figs. 4A and 4B). Under no netting with no organic acid application or applications of humic acid or glutamine, an irrigation interval of 2 d or 4 d, the crop produced the highest yield, while an irrigation interval of 6 d decreased the crop yield. However, with no netting and the application of arginine, the crop had the highest yield with an irrigation interval of 4 d. The crops growing under green netting with the application of glutamine or arginine; the crops had the highest yields at an irrigation interval of 4 d, whereas with the application of humic acid, the highest yield was with an irrigation interval of 2 d.

Table 1 Analysis of variance of effects of experimental factors on studied traits of strawberry

Source of variation	df	F-value								
		Yield	Phenolics	Antioxidant capacity	TSS	Taste index	Fruit protein	Peroxidase	Proline	Membrane stability index
Replication	2	1.13^{ns}	2.50^{ns}	1.11^{ns}	0.13^{ns}	0.56^{ns}	0.13^{ns}	4.32*	2.20^{ns}	0.53^{ns}
Netting	3	10.37**	5.73**	2.92*	10.89**	11.10**	0.96^{ns}	12.58**	1.08^{ns}	5.71**
Organic acid	3	6.19**	0.81^{ns}	1.21^{ns}	0.80^{ns}	1.77^{ns}	3.09*	436.47**	6.01**	4.59**
Netting × Organic acid	9	7.94**	2.50*	4.96**	1.90^{ns}	2.46*	1.18^{ns}	217.54**	2.56*	2.38*
Irrigation	2	33.38**	0.46^{ns}	0.84^{ns}	3.00^{ns}	2.37^{ns}	1.58^{ns}	45.11**	8.41**	8.12**
Netting × Irrigation	6	5.78**	0.69^{ns}	6.82**	2.63*	3.90**	1.22^{ns}	36.26**	3.19**	5.96**
Organic acid × Irrigation	6	5.28**	0.80^{ns}	3.34**	2.71*	3.70**	4.71**	93.11**	1.93^{ns}	2.30*
Netting × Organic acid × Irrigation	18	4.91**	1.12^{ns}	3.31**	1.40^{ns}	1.68^{ns}	1.87*	149.65**	1.87*	1.64^{ns}

df = degrees of freedom; TSS = total soluble solids.

^{ns} = not significant; * = significant at $p < 0.05$; ** = highly significant at $p < 0.01$.

Table 2 Comparison of main effect of netting on quantity and quality parameters of strawberries

Netting color	Yield (g/plant)	Phenolics (mg/ml)	Antioxidant capacity (DPPH %)	TSS (%)	Taste index (%)	Peroxidase (UNIT)*	Membrane stability index (%)
No netting	9.74±1.3^{c}	3.89±0.7^{c}	69.7±1.99^{a}	9.48±0.62^{b}	9.89±2.5^{c}	0.026±0.0018^{a}	80.43±2.22^{a}
Green	12.54±1.91^{b}	5.14±0.84^{ab}	65.00±1.56^{b}	9.28±0.60^{b}	10.06±2.68^{c}	0.025±0.0018^{ab}	79.82±1.96^{a}
Red	10.95±1.63^{bc}	5.88±0.86^{a}	66.7±1.7^{ab}	10.81±0.77^{a}	23.19±5.6^{a}	0.021±0.0015^{b}	74.86±1.64^{b}
Yellow	14.72±2.1^{a}	4.86±0.79^{b}	69.61±1.94^{a}	10.30±0.73^{a}	10.83±2.81^{b}	0.024±0.0017^{ab}	79.62±1.89^{a}

DPPH = 2,2-diphenylpicrylhydrazyl.

* = catalase and peroxidase expressed in micro moles of H_2O_2 consumed per minute per milligram of protein.

Mean ± SD values with different lowercase superscripts are significantly ($p < 0.05$) different.

Table 3 Comparison of main effects of organic acids on quantity and quality parameters of strawberries

Organic acid	Yield (g/plant)	Fruit protein (mg/g)	Peroxidase (units)*	Proline (mM/L)	Membrane stability index (%)
Control	11.13±1.23^{b}	0.15±0.09^{ab}	0.043±0.013^{a}	0.70±0.06^{b}	79.71±2.06^{a}
Humic acid	11.25±1.29^{b}	0.16±0.11^{a}	0.023±0.008^{b}	0.82±0.07^{a}	79.89±2.1^{a}
Glutamine	14.48±1.44^{a}	0.13±0.08^{b}	0.013±0.004^{c}	0.67±0.06^{b}	79.91±2.11^{a}
Arginine	11.08±1.11^{b}	0.14±0.09^{b}	0.017±0.006^{c}	0.68±0.06^{b}	75.22±1.98^{b}

* = peroxidase expressed in micro moles of H_2O_2 consumed per minute per milligram of protein.
Mean ± SD values with different lowercase superscripts are significantly ($p < 0.05$) different.

Table 4 Comparison of main effects of irrigation on quantity and quality parameters of strawberries

Irrigation interval (d)	Yield (g/plant)	Peroxidase (units)*	Proline (mM/L)	Membrane stability index (%)
2	12.91±1.53^{b}	0.020±0.003^{b}	0.66±0.05^{b}	81.57±3.02^{a}
4	14.77±2.24^{a}	0.025±0.002^{a}	0.70±0.046^{b}	76.33±2.76^{b}
6	8.27±2.71^{c}	0.027±0.001^{a}	0.79±0.034^{a}	78.15±1.98^{b}

* = peroxidase expressed in micro moles of H_2O_2 consumed per minute per milligram of protein.
Mean ± SD values with different lowercase superscripts are significantly ($p < 0.05$) different.

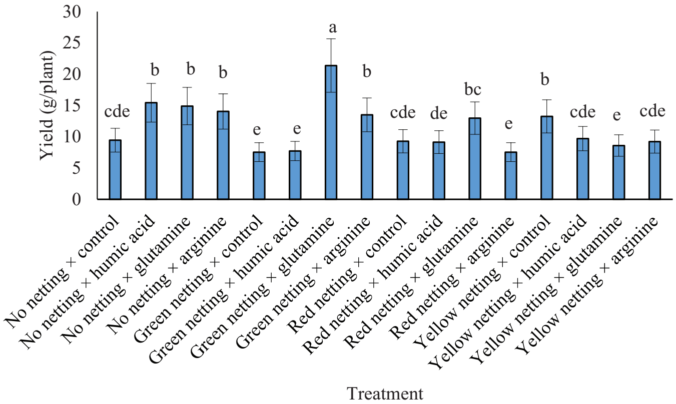

Fig. 1 Comparison of means for effect of 'netting × organic acid' on total fruit yield, where different lowercase letters above bars indicate significant ($p < 0.05$) differences among means and error bars represent ± SD

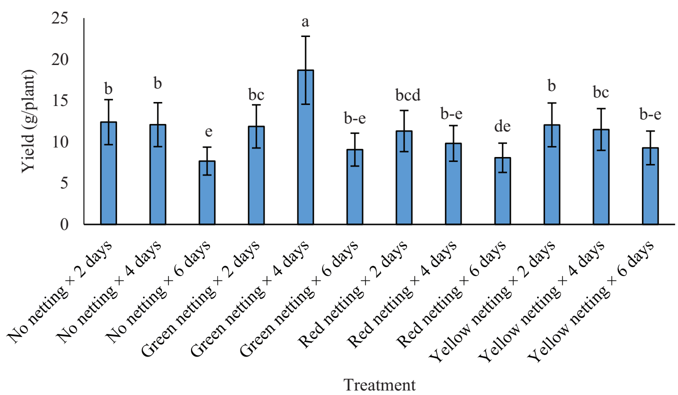

Fig. 2 Comparison of means for effect of 'netting × irrigation' on total fruit yield, where different lowercase letters above bars indicate significant ($p < 0.05$) differences among means and error bars represent ± SD

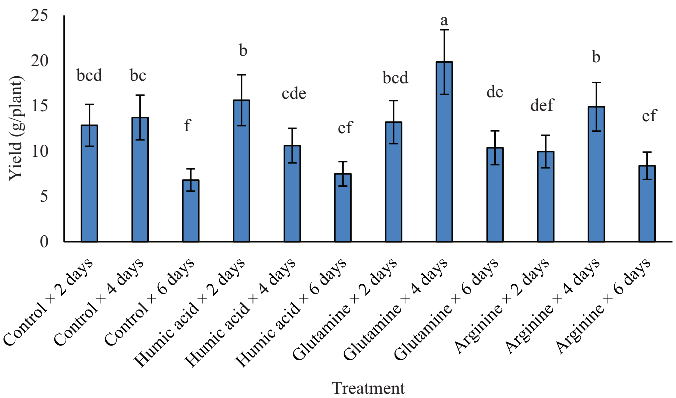

Fig. 3 Comparison of means for effect of 'organic acid × irrigation' on total fruit yield, where different lowercase letters above bars indicate significant ($p < 0.05$) differences among means and error bars represent ± SD

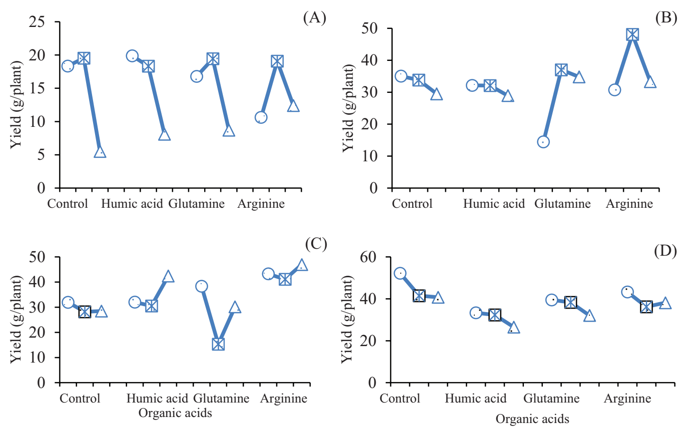

Fig. 4 Three-way interaction showing means yield interaction between two factors of organic acid applications and irrigation intervals of: (A) no netting; (B) Green netting; (C) Red netting; (D) Yellow netting; where o = 2 d, ж = 4 d and Δ = 6 d

Phenolics

The results of analysis of variance (ANOVA) showed that the main effect of shade netting and the interactive effect of 'shade netting × organic acids' were significant regarding the phenolics content, but the effects of other experimental treatments were not (Table 1). Among the netting types, the highest amount of phenolics was related to the red and green netting (5.88 ± 0.86 mg/L and 5.14 ± 0.84 mg/L, respectively) treatments and the lowest to the no-netting treatment (3.89 ± 0.71 mg/L), as shown in Table 2. For interaction data showed that under no netting, the highest amount of phenolics were with the application of any of the organic acids (humic, glutamine, arginine) and the lowest was in the treatment with no application of organic acid (3.03 ± 0.40 mg/mL), as shown in Table 5, whereas for the green, red and yellow netting types, the highest phenolic contents were without any organic acid application (Table 5). Overall, for the combination of netting color and application of organic acid, the crop under red netting with the application of humic acid had the highest yield (7.18 ± 0.54 mg/ml). Based on these results, the use of nets with organic acids always outperformed the control (no netting and without organic acids) regarding enhancing the strawberry phenolics content.

Antioxidant capacity

According to the ANOVA, the netting main effect and all the interactions were significant regarding the antioxidant capacity except for the main effect of organic acids and irrigation (Table 1). The highest antioxidant capacity was related to the treatments of no netting and yellow netting and the lowest to the green netting (65.00 ± 1.56%), as shown in Table 2. Regarding the interaction of 'netting × organic acids', the comparison of means showed that yellow netting (76.89 ± 5.00%) and 'no netting × humic acid' (76.88 ± 3.87%) were related to the highest and 'green netting × arginine' was related to the lowest antioxidant capacity (57.20 ± 2.62%), as shown in Table 5. The comparison of mean data for the interaction of 'netting × irrigation' (Table 6) revealed that the highest (78.40 ± 3.68%) and lowest (60.67 ± 2.76%) antioxidant capacities were obtained from 'no netting × 6 day irrigation interval' and 'green netting × 6 day irrigation interval'. Considering 'organic acid × irrigation', the plants treated with 'arginine × 2 days' had the highest (73.30 ± 2.56%) and those treated with 'arginine × 4 days' had the lowest (59.65 ± 1.76%) antioxidant capacities (Table 7). For the three-way interaction, the mean values for the antioxidant capacity interaction between the two factors of organic acid application and irrigation interval under different netting types are presented in Table 8. With no netting with the application of humic acid, the irrigation interval of 6 d produced the crops with the highest antioxidant capacity (84.39 ± 4.23%). for the three-way interaction of the three factors, the minimum antioxidant capacity was obtained for 'green netting × arginine × 4 days' (49.17 ± 2.12%), as shown in Table 8.

Peroxidase

Based on the ANOVA, the main and interactive effects of all experimental factors were significant regarding peroxidase (Table 1). The highest peroxidase activity (0.026 ± 0.0018 units) was related to the no-netting treatment and the lowest (0.021 ± 0.0015 units) to the red netting (Table 2). The highest peroxidase enzyme (0.043 ± 0.013 units) was obtained from the control treatment and the lowest (0.013 ± 0.004 units) from the treatment with glutamine (Table 3). Based on the comparison of means for irrigation, the plants with the irrigation interval of either 6 d or 4 d had the highest peroxidase activity (0.027 ± 0.001 units and 0.025 ± 0.002 units, respectively) and those irrigated every 2 d had the lowest (0.020 ± 0.003 units), as shown in Table 4. The highest amount of peroxidase (0.052 ± 0.001 units) was obtained from 'no netting × control' and the lowest (0.002 ± 0.000 units) from 'red netting × humic acid' and 'yellow netting × glutamine' (Table 5). Data for the interaction of 'netting × irrigation' indicated that the highest peroxidase activities (0.034 ± 0.003 units and 0.032 ± 0.003 units) were related to 'no netting × 6 days' and 'green netting × 4 days', respectively, and the lowest (0.014 ± 0.001 units) to 'red netting × 2 days' (Table 6). The interaction of 'netting × irrigation', showed that under no netting and red netting, the highest POD values were observed for the crops with an irrigation interval of 6 d (0.034 ± 0.003 unit and 0.029 ± 0.002 unit, respectively), as shown in Table 6, whereas under the green netting and yellow, the crops with an irrigation interval of 4 d had the highest POD values (0.032 ± 0.003 units and 0.024 ± 0.002 units). Overall, for the combination treatment of netting color and irrigation treatment, the crops under green netting and an irrigation interval of 4 d had the highest peroxidase activity (0.032 ± 0.003 units), as shown in Table 6. For the interaction of 'organic acids × irrigation' (Table 7), the 'control × 4 days' treatment had the highest peroxidase activity (0.057 ± 0.005 units) and 'glutamine × 4 days' (0.005 ± 0.001 units) and 'arginine × 2 days' (0.008 ± 0.001 units) had the lowest.

Table 5 Comparison of effects of 'netting × organic acid' on quantity and quality parameters of strawberries

Treatment		Phenolics	Antioxidant capacity	Taste index	Membrane stability	Peroxidase	Proline
Netting color	Organic acid	(mg/mL)	(DPPH%)	(%)	index (%)	(units)*	(mM/L)
No netting	Control	3.03±0.40^{h}	60.95±5.3^{ef}	10.66±0.86^{cde}	78.94±4.01^{abcd}	0.052±0.001^{a}	0.63±0.03^{cde}
	Humic acid	4.96±0.56^{bcdefg}	76.88±3.87^{a}	10.16±1.03^{def}	84.13±3.45^{a}	0.038±0.001^{bc}	0.71±0.0021^{bcd}
	Glutamine	3.55±0.66^{gh}	67.49±4.09^{bcde}	9.61±2.11^{efg}	79.32±2.78^{abcd}	0.012±0.000^{de}	0.75±0.00^{abcd}
	Arginine	4.039±0.52^{cdefgh}	73.78±3.45^{ab}	9.11±1.45^{fg}	79.34±3.51^{abcd}	0.004±0.000^{ef}	0.70±0.001^{bcde}
Green	Control	5.94±0.95^{abc}	71.29±3.51^{abc}	10.57±0.79^{cdef}	79.65±2.70^{abcd}	0.048±0.001^{a}	0.73±0.002^{abcd}
	Humic acid	3.76±0.43^{fgh}	68.22±4.21^{bcde}	8.57±0.95^{g}	82.89±2.41^{ab}	0.044±0.002^{ab}	0.84±0.000^{ab}
	Glutamine	5.52±0.61^{abcdef}	63.29±3.75^{def}	10.70±1.04^{cde}	81.96±2.31^{abc}	0.006±0.000^{ef}	0.55±0.004^{e}
	Arginine	5.34±0.97a^{bcdefg}	57.20±2.62^{f}	10.39±1.53^{cdef}	74.78±4.31^{de}	0.004±0.000^{ef}	0.64±0.004^{cde}
Red	Control	6.313±1.31^{ab}	64.24±3.50^{cef}	10.65±1.17^{cde}	78.23±2.84^{abcd}	0.033±0.004^{c}	0.63±0.004^{cde}
	Humic acid	7.18±0.54^{a}	67.48±3.42^{bcde}	11.70±1.74^{c}	70.38±2.42^{e}	0.002±0.000^{f}	0.87±0.001^{a}
	Glutamine	5.25±1.06^{bcdefg}	69.51±3.86^{abcd}	28.32±2.70^{b}	80.27±3.12^{abcd}	0.034±0.001^{c}	0.77±0.002^{abc}
	Arginine	4.77±0.84^{bcdefgh}	65.91±2.80^{cde}	42.10±2.03^{a}	70.55±4.21^{e}	0.017±0.002^{d}	0.70±0.003^{bcd}
Yellow	Control	5.86±1.00^{abcd}	76.89±5.00^{a}	10.32±3.07^{cdef}	82.00±2.83^{abc}	0.038±0.001^{bc}	0.82±0.004^{ab}
	Humic acid	3.87±0.46^{efgh}	65.97±3.47^{cde}	10.91±1.06^{cd}e	82.16±3.40^{abc}	0.009±0.000^{def}	0.85±0.000^{ab}
	Glutamine	5.72±0.74^{abcde}	66.85±2.66^{bcde}	11.55±2.08^{cd}	78.08±2.81^{bcd}	0.002±0.000^{f}	0.59±0.003^{de}
	Arginine	4.01±0.12^{defh}	68.74±4.10^{bcd}	10.53±0.91^{cdef}	76.23±3.07^{cde}	0.044±0.003^{ab}	0.69±0.001^{bcde}

DPPH = 2,2-diphenylpicrylhydrazyl.

* = peroxidase expressed in micro moles of H_2O_2 consumed per minute per milligram of protein.

Mean ± SD values with different lowercase superscripts are significantly ($p < 0.05$) different.

Table 6 Comparison of effects of 'netting × irrigation' on quantity and quality parameters of strawberries

Treatment		Antioxidant capacity	TSS	Taste index	Membrane stability	Peroxidase	Proline
Netting color	Irrigation interval (d)	(DPPH%)	(%)	(%)	index (%)	(units)*	(mM/L)
No netting	2	63.73±3.45^{de}	9.07±.92de	9.19±0.95^{d}	80.45±4.32^{abc}	0.017±0.001^{de}	0.69±0.004^{bc}
	4	67.19±3.24^{bcde}	9.00±0.89^{de}	9.44±1.02^{d}	83.94±3.88^{a}	0.028±0.002^{abc}	0.74±0.005^{abc}
	6	78.40±3.68^{a}	10.38±1.03^{abc}	11.03±1.98^{bc}	76.91±3.48^{cd}	0.034±0.003^{a}	0.67±0.003^{bcd}
	2	71.02±3.98^{bc}	9.09±1.01^{de}	10.17±1.85^{cd}	79.46±3.83^{abcd}	0.022±0.002^{bcde}	0.84±0.006^{a}
Green	4	63.31±2.85^{e}	8.71±0.85^{e}	9.10±1.05^{d}	76.54±3.09^{cd}	0.032±0.003^{a}	0.69±0.003^{bc}
	6	60.67±2.76^{e}	10.04±1.22^{bcd}	10.90±1.42^{bc}	83.47±4.23^{a}	0.023±0.001^{bcd}	0.53±0.002^{d}
	2	71.16±2.98^{bc}	10.89±1.43^{ab}	11.66±1.88^{b}	82.16±3.78^{ab}	0.014±0.001^{e}	0.80±0.006^{ab}
Red	4	66.29±2.80^{bcde}	11.27±1.86^{a}	46.72±2.24^{a}	68.04±2.45^{e}	0.021±0.002^{cde}	0.76±0.005^{ab}
	6	62.90±2.56^{e}	10.25±1.24^{abc}	11.2±1.43b^{c}	74.37±3.32^{d}	0.029±0.002^{ab}	0.67±0.003^{bc}
Yellow	2	70.14±3.58^{bcd}	10.42±1.54^{abc}	11.65±1.87^{b}	84.19±4.56^{a}	0.021±0.002^{bcde}	0.84±0.006^{a}
	4	72.79±3.86^{ab}	9.84±0.92^{cd}	10.09±1.23^{cd}	76.82±3.54^{cd}	0.019±0.01^{de}	0.62±0.003^{cd}
	6	65.91±2.96^{cde}	10.6±1.13^{abc}	10.75±1.67^{bc}	77.84±3.68^{bcd}	0.024±0.002^{bcd}	0.75±0.004^{abc}

DPPH = 2,2-diphenylpicrylhydrazyl; TSS = total soluble solids.

* = peroxidase expressed in micro moles of H_2O_2 consumed per minute per milligram of protein.

Mean ± SD values with different lowercase superscripts are significantly ($p < 0.05$) different.

Table 7 Comparison of effects of ‘organic acids × irrigation’ on quantity and quality parameters of strawberries

Treatment		Antioxidant capacity	TSS	Taste index	Fruit protein	Peroxidase	Membrane stability
Organic acid	Irrigation interval (d)	(DPPH%)	(%)	(%)	(mg/g)	(units)*	index (%)
Control	2	68.04±1.95^{ab}	8.89±0.91^{d}	10.79±1.54^{cde}	0.135±0.07^{cd}	0.028±0.003^{cd}	80.81±4.66^{ab}
	4	70.56±2.23^{ab}	9.73±0.98^{bcd}	9.87±1.34^{ef}	0.167±0.08b^{c}	0.057±0.005^{a}	77.27±3.97^{bc}
	6	66.43±1.87^{b}	10.53±1.14^{ab}	10.99±1.56^{cde}	0.172±0.09^{ab}	0.043±0.004^{b}	81.03±4.74^{ab}
Humic acid	2	70.16±2.32^{ab}	9.82±0.92^{acd}	9.81±1.23^{ef}	0.203±0.09^{a}	0.031±0.003^{c}	83.03±4.82^{a}
	4	69.30±2.02^{a}b	9.37±1.03^{cd}	9.30±1.11^{f}	0.146±0.07^{bc}	0.018±0.002^{ef}	77.30±3.86^{bc}
	6	69.45±1.98^{ab}	10.86±1.24^{a}	11.89±1.89^{c}	0.141±0.06^{bc}	0.021±0.002^{def}	79.34±3.98^{abc}
Glutamine	2	64.54±2.01^{bc}	10.43±1.21^{ab}	11.65±1.76^{cd}	0.146±0.06^{bc}	0.013±0.001^{fg}	81.09±4.86^{ab}
	4	70.08±2.34^{ab}	10.55±1.35^{ab}	23.15±2.02^{b}	0.161±0.07^{bc}	0.005±0.001^{g}	81.20±4.93^{ab}
	6	65.73±1.89^{bc}	9.78±0.95^{bcd}	10.34±1.32^{ef}	0.104±0.05^{d}	0.022±0.002^{de}	77.44±4.03^{bc}
Arginine	2	73.30±2.56^{a}	9.83±0.96^{abcd}	10.41±1.44^{def}	0.135±0.06^{cd}	0.008±0.001^{g}	81.33±4.94^{ab}
	4	59.65±1.76^{c}	9.65±0.94^{bcd}	33.02±2.21^{a}	0.141±0.06^{bc}	0.019±0.002^{ef}	69.57±3.52^{d}
	6	66.28±2.10^{b}	10.14±1.06^{abc}	10.67±1.47^{cde}	0.146±0.07^{bc}	0.02±0.002^{cde}	74.77±3.76^{cd}

DPPH = 2,2-diphenylpicrylhydrazyl.; TSS = total soluble solids

* = peroxidase expressed in micro moles of H_2O_2 consumed per minute per milligram of protein.

Mean ± SD values with different lowercase superscripts are significantly ($p < 0.05$) different.

Table 8 Comparison of effects of ‘netting ×organic acids × irrigation’ on quantity and quality parameters of strawberries

Colored netting	Organic acid	Irrigation interval (d)	Antioxidant capacity (DPPH%)	Fruit protein (mg/g)	Peroxidase (units)*	Proline (mM/l)
No netting	Control	2	54.81±2.56^{oqr}	0.10±0.08^{def}	0.008±0.001^{ijkl}	0.48±0.04^{klmn}
		4	57.13±2.43^{nopqr}	0.15±0.09^{bcde}	0.085±0.003^{a}	0.83±0.07^{abcdefg}
		6	70.90±3.12^{bcdefghijklm}	0.17±0.10^{abcd}	0.062±0.002^{bcd}	0.59±0.05^{fghijklmn}
No netting	Humic acid	2	66.74±2.65^{efghijklmnop}	0.19±0.11^{abc}	0.056±0.002^{cde}	0.76±0.06^{bcdefghij}
		4	79.51±3.54^{abcde}	0.17±0.09^{abcd}	0.007±0.004^{jk}l	0.72±0.06^{cdefghkl}
		6	84.39±4.23^{a}	0.14±0.08^{bcde}	0.051±0.003^{de}	0.64± 0.05^{efghijklmn}
No netting	Glutamine	2	51.72±2.63^{qr}	0.14±0.08^{bcde}	0.001±0.0001 ^{l}	0.73±0.06^{cdefghijkl}
		4	69.45±3.12^{cdefghijklmn}	0.17±0.09^{abcd}	0.014±0.001^{hijkl}	0.82±0.07^{abcdefg}
		6	81.30±3.46^{abc}	0.06±0.05^{f}	0.020±0.002^{ghij}	0.69±0.07^{defghijklm}
No netting	Arginine	2	81.65±3.59^{abc}	0.10±0.08^{def}	0.004±0.002^{kl}	0.78±0.06^{bcdefghji}
		4	62.67±3.03^{hijklmnopq}	0.14±0.07^{bcde}	0.005±0.001^{jkl}	0.59±0.04^{fghijklmn}
		6	77.02±2.88^{abcdefg}	0.14±0.09^{bcde}	0.003±0.001^{kl}	0.74±0.07^{cdefghk}
Green netting	Control	2	69.37±2.58^{cdefghijklmn}	0.17±0.10^{abcd}	0.007±0.002^{jkl}	0.97±0.08^{abc}
		4	83.85±2.98^{ab}	0.14±0.07^{bcde}	0.065±0.003^{bcd}	0.80±0.07^{abcdefgh}
		6	60.66±2.44^{jklmnopqr}	0.19±0.12^{abc}	0.072±0.002^{ab}	0.43±0.05^{mn}
Green netting	Humic acid	2	77.70±3.87^{abcdef}	0.21±0.14^{ab}	0.058±0.003^{bcde}	0.95±0.08^{abcd}
		4	63.34±3.01^{hijklmnopq}	0.19±0.09^{abc}	0.061±0.002^{bcde}	0.88±0.07^{abcde}
		6	63.63±2.64^{hijklmnopq}	0.14±0.07^{bcde}	0.012±0.001^{hijkl}	0.68±0.05^{defghijklm}

Table 8 Continued

Colored netting	Organic acid	Irrigation interval (d)	Antioxidant capacity (DPPH%)	Fruit protein (mg/g)	Peroxidase (units)*	Proline (mM/l)
Green netting	Glutamine	2	$72.65\pm2.95^{abcdefghijkl}$	0.14 ± 0.08^{bcde}	0.015 ± 0.001^{hijkl}	0.75 ± 0.06^{bcdefghijk}
		4	56.90 ± 2.52^{nopqr}	0.12 ± 0.06^{cdef}	0.002 ± 0.001^{l}	0.39 ± 0.04^{n}
		6	60.33 ± 3.03^{klmnopqr}	0.12 ± 0.08^{cdef}	0.002 ± 0.000^{l}	0.50 ± 0.04^{jklmn}
Green netting	Arginine	2	$64.37\pm2.45^{ghijklmnopq}$	0.17 ± 0.09^{abcd}	0.006 ± 0.002^{jkl}	$0.70\pm0.06^{cdefghijklm}$
		4	49.17 ± 2.12^{r}	0.17 ± 0.08^{abcd}	0.002 ± 0.001^{l}	0.69 ± 0.05^{defghijklm}
		6	58.07 ± 2.98^{mnopqr}	0.10 ± 0.07^{def}	0.005 ± 0.002^{jkl}	0.52 ± 0.05^{ijklmn}
Red netting	Control	2	$68.87\pm2.09^{cdefghijklmn}$	0.08 ± 0.05^{ef}	0.012 ± 0.001^{hijkl}	0.66 ± 0.06^{efghijklm}
		4	$63.29\pm2.76^{hijklmnopq}$	0.23 ± 0.13^{a}	0.053 ± 0.002^{de}	0.58 ± 0.05^{fghijklmn}
		6	60.55 ± 2.62^{jklmnopqr}	0.19 ± 0.12^{abc}	0.034 ± 0.001^{fg}	0.60 ± 0.07^{efghijklmn}
Red netting	Humic acid	2	60.95 ± 3.14^{jklmnopqr}	0.23 ± 0.14^{a}	0.003 ± 0.001^{kl}	0.77 ± 0.08^{bcdefghij}
		4	74.33 ± 3.65^{abcdefghi}	0.12 ± 0.09^{cdef}	0.002 ± 0.000^{l}	1.02 ± 0.09^{ab}
		6	$67.17\pm2.98^{efghijklmno}$	0.15 ± 0.08^{bcde}	0.001 ± 0.000^{l}	0.83 ± 0.07^{abcdef}
Red netting	Glutamine	2	$73.49\pm4.03^{abcdefghijk}$	0.15 ± 0.08^{bcde}	0.034 ± 0.001^{fg}	1.06 ± 0.08^{a}
		4	$73.56\pm2.82^{abcdefghij}$	0.17 ± 0.09^{abcd}	0.004 ± 0.001^{kl}	0.73 ± 0.6^{cdefghijkl}
		6	$61.48\pm3.03^{ijklmnopqr}$	0.08 ± 0.06^{ef}	0.063 ± 0.003^{bcd}	0.53 ± 0.05^{hijklmn}
Red netting	Arginine	2	81.33 ± 3.08^{abc}	0.10 ± 0.07^{def}	0.00 ± 0.000^{jkl}	0.70 ± 0.06^{cdefghijkl}
		4	54.00 ± 2.58^{pqr}	0.12 ± 0.09^{cdef}	0.024 ± 0.001^{ghi}	0.74 ± 0.06^{cdefghijk}
		6	62.41 ± 2.52^{ijklmnopq}	0.15 ± 0.07^{bcde}	0.019 ± 0.001^{ghijk}	0.67 ± 0.05^{efghijklm}
Yellow netting	Control	2	79.12 ± 3.95^{abcde}	0.19 ± 0.13^{abc}	0.086 ± 0.003^{a}	0.91 ± 0.07^{abcde}
		4	77.96 ± 2.58^{abcdef}	0.15 ± 0.08^{bcde}	0.025 ± 0.002^{gh}	0.70 ± 0.07^{defghijklm}
		6	$73.60\pm3.44^{abcdefghij}$	0.15 ± 0.07^{bcde}	0.004 ± 0.001^{jkl}	0.85 ± 0.08^{abcdef}
Yellow netting	Humic acid	2	75.26 ± 4.01^{abcdefgh}	0.19 ± 0.12^{abc}	0.007 ± 0.001^{jkl}	1.01 ± 0.08^{ab}
		4	60.03 ± 3.08^{lmnopqr}	0.10 ± 0.06^{def}	0.003 ± 0.001^{kl}	0.83 ± 0.07^{abcdef}
		6	$62.62\pm2.98^{hijklmnopq}$	0.12 ± 0.09^{cdef}	0.019 ± 0.001^{ghijk}	0.70 ± 0.06^{cdefghijkl}
Yellow netting	Glutamine	2	60.31 ± 2.23^{lmnopqr}	0.15 ± 0.10^{bcde}	0.002 ± 0.000^{l}	0.73 ± 0.07^{cdefghijkl}
		4	80.42 ± 4.18^{abcd}	0.19 ± 0.09^{abc}	0.001 ± 0.000^{l}	0.51 ± 0.04^{jklmn}
		6	59.81 ± 2.32^{lmnopqr}	0.15 ± 0.10^{bcde}	0.001 ± 0.000^{l}	0.56 ± 0.05^{ghijklmn}
Yellow netting	Arginine	2	$65.8\pm3.04^{fghijklmnop}$	0.17 ± 0.09^{abcd}	0.016 ± 0.001^{hijkl}	0.73 ± 0.06^{cdefghijkl}
		4	$72.77\pm 3.17^{abcdefghijkl}$	0.12 ± 0.08^{cdef}	0.045 ± 0.002^{ef}	0.46 ± 0.5^{lmn}
		6	$67.60\pm2.88^{defghijklmno}$	0.19 ± 0.13^{abc}	0.071 ± 0.003^{abc}	0.90 ± 0.08^{abcde}

DPPH = 2,2-diphenylpicrylhydrazyl.

* = peroxidase expressed in micro moles of H_2O_2 consumed per minute per milligram of protein.

Mean ± SD values with different lowercase superscripts are significantly ($p < 0.05$) different.

For the three-way interaction, the treatment with no netting and no organic acid application and an irrigation interval of 4 d had the highest POD (0.085 ± 0.003 units) and when the irrigation interval was 2 d, the crop POD decreased (0.008 ± 0.001 units), whereas with no netting and the application of glutamine, the crop had the lowest POD with an irrigation interval of 2 d (0.001 ± 0.000l units). The crops grown under green netting with the application of glutamine or arginine had the lowest POD with irrigation intervals of 4 d or 6 d, whereas with the application of humic acid, the crop had the lowest POD with an irrigation interval of 6 d (0.012 ± 0.001 units), as shown in Table 8. The highest peroxidase content (0.085 ± 0.003 units) was observed for the treatment 'no netting × control × 4 days' and the lowest for 'no netting × glutamine × 2 days', 'yellow netting × glutamine × 2 d, 4 d or 6 d' (Table 8).

Membrane stability index

The ANOVA revealed that the effects of all treatments were significant regarding the membrane stability index (MSI), except the three-way interaction (Table 1). Based on the comparison of means, the highest MSI was related to no netting (80.43 ± 2.22%), green netting (79.82 ± 1.96%) and yellow netting (79.62 ± 1.89%). The lowest MSI (74.86 ± 1.64%) was obtained from the red netting (Table 2). The plants treated with glutamine had the highest MSI (79.91 ± 2.11%) and those treated with arginine had the lowest MSI (74.86 ± 1.64%), as shown in Table 3. Among the irrigation intervals 2 d and 6 d were associated with the highest (81.57 ± 3.02%) and lowest (76.33 ± 2.76%) MSI values, respectively (Table 4). The interaction of 'no netting × humic acid' produced the highest MSI (84.13 ± 3.45%), and the interactions of 'red netting × humic acid' and 'red netting × arginine' produced the lowest MSI (70.38 ± 2.42 and 70.55 ± 4.21%), as shown in Table 5. The interaction of 'netting × irrigation' indicated that 'no netting × 4 days' (83.94 ± 3.88%), 'green netting × 6 days' (83.47 ± 4.23%), and 'yellow netting × 2 days' (84.19 ± 4.56%) were related to the highest MSI and 'red netting × 4 days' (68.04±2.45%) was related to the lowest MSI (Table 6). Finally, for the interaction between organic acids and irrigation, the highest MSI (83.03 ± 4.82%) was obtained from 'humic acid × 2 days' and the lowest (69.57 ± 3.52%) from 'arginine × 4 days' (Table 7). All treatments of organic acids with an irrigation interval of 2 d had the highest MSI values (Table 7).

Total soluble solids and taste index

The results of ANOVA showed that the main effect of netting and the interaction of 'netting × irrigation' and 'organic acids× irrigation' were significant regarding TSS. However, the other main effect and interactions did not significantly influence TSS (Table 1). The highest TSS was related to the red netting (10.81 ± 0.77%) and yellow netting (10.30 ± 0.73%), as shown in Table 2. Data on the interaction of 'netting × irrigation' revealed that the highest TSS (11.27 ± 1.86%) was obtained from 'red netting × 4 days' and the lowest (8.71 ± 0.85%) from 'green netting × 4 days' (Table 6). Based on the comparison of mean data for the interaction of 'organic acids × irrigation', the highest and lowest TSS levels were produced by the interactions of 'humic acid × 6 days' and 'control × 2 days', (10.86 ± 1.24 and 8.89 ± 0.91%, respectively), as shown in Table 7. Based on the ANOVA, the main effect of netting and the interactive effects of 'netting × organic acids', 'netting × irrigation' and 'organic acids × irrigation' were significant regarding the taste index (Table 1). The highest taste index (23.19 ± 5.6%) was observed in the plants grown under red netting and the lowest (9.89 ± 2.5%) in those grown with no netting (Table 2). Data on the interaction of 'netting × organic acid' (Table 5) revealed that the highest taste index (42.10 ± 2.03%) was related to 'red netting × arginine' and the lowest index (8.57 ± 0.95%) to 'green netting × humic acid' (Table 5). The difference between the two treatment combinations was more than 4.9 times. According to the data on 'netting × irrigation', the plants grown under 'red netting × 4 days' had the highest fruit taste index (46.72 ± 2.24%) and those grown under 'green netting × 4 days' (9.10 ± 1.05%), 'no netting × 2 days' (9.19 ± 0.95%) or 'no netting × 4 days' (9.44 ± 1.02%) had the lowest (Table 6).

Fruit protein

The ANOVA revealed significant effects of organic acids, 'organic acid × irrigation' and 'netting × organic acid × irrigation' regarding fruit protein (Table 1). The highest fruit protein content was obtained from the application of humic acid and the lowest from the treatment of glutamine (Table 3). Based on the comparison of means for the interaction of 'organic acid × irrigation', the highest fruit protein content was observed in the interaction of 'humic acid × 2 days' and the lowest in the treatment of 'glutamine × 6 days' (Table 7). Regarding the three-way effect, the highest fruit protein was related to 'red netting × control × 4 days' and 'red netting × humic acid × 2 days' and the lowest to 'no netting × glutamine × 6 days' (Table 8).

Proline content

The effects of all main and interaction effects, except the main effect of netting and the interactive effect of 'organic acids × irrigation', were significant regarding the proline content (Table 1). Based on the comparison of the mean data, the highest proline content (0.82 ± 0.07 mM/L) was obtained from the treatment of humic acid (Table 3). Among the irrigation levels, the highest proline content (0.79 ± 0.034 mM/L) was observed in the plants with an irrigation interval of 6 d and the lowest in those irrigated every 2d (0.66 ± 0.05 mM/L) and 4 d (0.70 ± 0.046 mM/L), as shown in Table 3. Comparison of the mean data of the trilateral effect of 'netting × organic acids × irrigation' revealed that 'red netting × glutamine × 2 days' was related to the highest proline content (1.06 ± 0.08 mM/L) and 'green netting × glutamine × 4 days' was related to the lowest (0.39 ± 0.04 mM/L), as shown in Table 8.

Discussion

In the present study, strawberry plants grown under netting had higher yields than under no netting and among the treatments under netting, the highest fruit yield was under the yellow netting. The impact of colored netting is related to the greater filtration of sunlight along with the physical protection of the crops. The colored nets absorb UV, blue, green, yellow, red, far-red or near-infrared wavelengths. The radiation spectrum is mostly manipulated to strengthen and stimulate plant physiological responses through photosynthetic and photomorphological processes (Shahak et al., 2008b). The main effect of colored nets is on the reduction of Photosynthetically active radiation (PAR) availability. Moderate shading would reduce plant radiation and heat and water stress and increase gas exchange and the availability of carbohydrates for fruit and tree growth (Bastías and Corelli-Grappadelli, 2012). In strawberries, it seems that the PAR availability under the yellow net was greater than for the other net types tested, resulting in a greater yield.

Of the organic acids tested, the application of glutamine produced the highest yield whereas for the irrigation interval, 4 d produced the highest yield. As amino acids are the precursors used during chlorophyll synthesis, exogenous amino acids application may affect dry matter production in plants (Yaronskaya et al., 2006). In particular, glutamic acid has been reported to increase strawberry plant dry weight under abiotic stress conditions (Mondal et al., 2013). Furthermore, the foliar application of amino acids increases the plant protein contents, which ultimately increases the dry matter (Das et al., 2002). Talukder et al. (2018) reported that spraying glutamic acid had a positive influence on the growth and yield of strawberry plants. Strawberry is an example of a water-intensive crop that is exacerbated because the plants have a shallow rooting system, high leaf area and a large fruit water content (Ariza et al., 2021).

The two-way and three-way interactive effects of 'netting x organic acid', 'netting × irrigation' and 'netting × organic acid × irrigation' revealed that the highest yield was obtained from the application of green netting along with glutamic acid (21.38 ± 2.24 g/plant), with an irrigation interval of 4 d (18.68 ± 2.11g/plant) and green netting with application of glutamine and irrigated every 4 days, (40.80 ± 2.41 g/plant), implying the effectiveness of colored netting on increasing the yield. When glutamic acid is applied during flowering, crop production can be increased due to its effect on pollination and pollen germination (Cao et al., 2010). Koukounaras et al. (2013) reported the positive impact of the application of amino acids on tomato yield. The lowest yield in the present study was observed with an irrigation interval of 6 d. The decline in yield due to water deficit could be associated with the inadequacy of soil moisture in the root zone, which reduces various physiological processes, including nutrient uptake, plant growth, photosynthesis and dry matter accumulation, resulting in lower yields under water stress (Simsek and Comlekcioglu, 2011).

The biosynthesis of most phytochemicals depends on the light quantity and quality, as observed in lettuce growing under black netting (Ntsoane, 2015). Blue light was effective in phenol synthesis in red amaranth cv. 'Roctoalta' (Khandaker et al., 2010). Organic acids, such as humic acid, have been reported to act as precursors of plant activators as well as secondary compounds, thereby increasing total phenolics (Viti et al., 1990). Antioxidant activity was higher in coriander leaves grown under yellow or red nets, while there were no significant differences in the antioxidant contents of the leaves grown under different nets (Buthelezi et al., 2016). Kong et al. (2013) found that the antioxidants of peppermint fruits increased under yellow netting. Abbasnia Zare et al. (2020) reported that the highest antioxidant levels of *Codiaeum variegatum* and *Aglaonema commutatum* were observed under green and control netting and the lowest under yellow netting.

Plant responses to increasing antioxidant capacity differ under different colored nets due to different light spectra (Miller et al., 2010). Mozafari et al. (2017) reported that the antioxidant activity of purapole increased with an increasing level of humic acid.

The application of yellow netting may reduce oxidative stresses, so a lower amount of peroxidase is synthesized to neutralize free oxygen radicals induced by oxidative stresses. Abbasnia Zare et al. (2020) reported that the highest peroxidase activity was obtained from ‘no netting × Aglaonema’ and the lowest from ‘red netting × Croton’. Thus, the escalation of the catalase and peroxidase contents in plant species plays a key role in inhibiting the accumulation of hydrogen peroxide in plants.

The preservation of membrane stability in stressful conditions reflects a control mechanism for drought tolerance. The increased stability of cell membranes due to the application of humic acid may be related to its direct hormonal effect or its indirect effect on increasing calcium uptake, which contributes to the mechanical resistance of the cell wall and the greater stability of the cell membrane (Nikbakht et al., 2008). Furthermore, humic acid improved the water status in bean plants by increasing the nutrient uptake and root cell permeability, thereby enhancing cell membrane stability under salinity stress (Semida et al., 2014). Based on the comparison of mean data for the interaction of ‘organic acids × irrigation’, the highest and lowest TSS values were produced by the interactions of ‘humic acid × 6 days’ and ‘control × 2 days’, respectively (Table 8). Application of humic acid increased TSS and titratable acidity (TA) in guava fruits (Rocha et al., 2016). It has been reported that the application of glutamine alone or along with humic acid increased sugar accumulation in strawberry fruits (Saied et al., 2005). In general, increasing TSS in drought-affected strawberries is a technique used by the plant to regulate osmosis and tolerate stress (Gine-Bordonaba and Terry, 2016). The decrease in TSS under stress can be ascribed to possible photosynthetic problems and a decrease in photosynthate accumulation (Bushra et al., 2014). Among the interactions of organic acids and irrigation intervals, the interactions of ‘arginine × 4 days’ and ‘humic acid × 4 days’ were related to the highest and lowest taste indices, respectively (Table 7). The TSS/TA ratio reaches an optimal level as the fruit loses less water (Ashournejad et al., 2010). With the use of organic acid, TSS increased and TA decreased, resulting in an improvement in the taste index of apricot fruits (Fathy et al., 2010).

The foliar application of free amino acids can be considered an important source for protein synthesis in plants (Raeisi et al., 2014). The application of compounds containing free amino acids increased the protein content of chickpea by increasing the plant nitrogen content (Mahmoudi, 2012). Abdel-Mawgoud et al. (2011) showed that the foliar application of amino acids and micronutrients on chickpeas increased their protein content, thereby increasing their yield significantly. Sanjari et al. (2015) reported that the foliar application of humic acid increased photosynthesis and the synthesis of carbohydrates and proteins in roselle. Water decline in plant tissues causes growth reduction, stomatal closure, photosynthesis reduction, degradation of proteins and changes in physiological processes (Ahmadi and Siosemardeh, 2009). Protein reduction under drought stress can be attributed to decreased synthesis of proteins or protein decomposition or both due to the increased activity of protease enzymes (Mafakheri et al., 2010).

Klamkowski and Treder (2008) reported decreases in biomass and leaf area in drought-exposed strawberries. Humic acid significantly increased the amount of proline in safflower plants exposed to different irrigation regimes (Karimi et al., 2016). Severe drought stress in strawberries led to a significant increase in proline productionand severe stress had a more negative effect on the proline, carbohydrates and chlorophyll contents (Ghaderi and Siosemarde, 2013).

Based on the present results, colored nets had desirable effects on the studied traits, so that the highest phenolics, antioxidant capacity, TSS, taste index, and membrane stability index (MSI) were obtained using colored netting. The highest proline content and MSI were observed in the plants treated with humic acid; the highest yield and MSI were observed in those treated with glutamine. Based on the results for the three-way interaction of ‘netting × organic acid × irrigation’, ‘green netting × glutamine × 4 days’ had the highest fruit yield, ‘no netting × humic acid × 6 days’ had the highest antioxidant capacity, ‘red netting × control × 4 days’ and ‘red netting × humic acid × 2 days’ had the highest peroxidase activity and fruit protein content, and ‘red netting × glutamine × 2 days’ had the highest proline content. In addition, irrigation intervals of 2 d or 4 d had more optimal effects on the studied traits than an interval of 6 d.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors thank their colleagues from Rasht Branch, Islamic Azad University, Rasht, Iran.

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